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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(54) Title:</b> LYMPHOCYTE INHIBITION BY HLA PEPTIDES  <b>(57) Abstract</b>  Novel peptides are provided for modulating the cytotoxic effect of CTL's. Novel peptides cross reactive with the C-terminal or N-terminal portions of the $\alpha$ , or $\alpha 2$ domains, respectively, of the MHC antigen providing the CTL restriction are employed for CTL modulation. The peptides may be used <i>in vivo</i> or <i>in vitro</i> for controlling cellular lysis.		

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LYMPHOCYTE INHIBITION BY HLA PEPTIDES

5

10

FIELD OF THE INVENTION

HLA peptide compositions affect T-cell activity subject to MHC restriction. Methods and compositions are provided involving the modulation of cytotoxicity toward allogeneic cells.

15

BACKGROUND OF THE INVENTION

Cytotoxic T-cells, particularly cytotoxic T-lymphocytes (CTL), are restricted in their activity by recognizing a specific major histocompatibility (MHC) antigen on the surface of the target cell, as well as an antigen exogenous to the host. The foreign antigen may be as a result of transplantation, viral infection, mutation, or the like. The presence of the determinant or restricting site of the MHC protein appears to be essential to the attack by the CTL against the cell carrying the foreign antigen. In this way, the immune system is able to destroy cells in the body, which, if otherwise allowed to proliferate, would result in the proliferation of pathogens or neoplastic cells.

20

25

30

The CTL's are also effective in destroying transplants of various organs or tissue which come from allogeneic hosts. The methods or treatments employed for protecting the transplanted cells frequently result

in the incapacitating of the immune system, subjecting the transplant recipient to opportunistic infection.

It is, therefore, of substantial interest to  
5 be able to modulate the cytotoxic system in a host to selectively inhibit a particular population of T-cells from performing their normal physiological function. In this manner, the immune system is substantially maintained intact, while a particular T-cell target may  
10 be protected.

#### DESCRIPTION OF THE RELEVANT LITERATURE

Clayberger et al., J. Exp. Med. (1985)  
11:1709-1714 describe HLA-A2 antigen in comparisons with  
15 HLA-Aw68 and Aw69. Townsend et al., Cell, (1986)  
44:959-968 suggests that CTL recognize segmental epitopes of denatured or degraded proteins in a similar way as helper T-cells. Holmes and Parham, EMBO J.  
(1985) 4:2849-2854 describe the relationship of HLA-A2.  
20 Aw68 and Aw69. CTL target specificity has been taught to be extremely sensitive to changes in structure of human Class I molecules (Durna and Pease, Transplantation, (1986) 41:279-285; Biddison, et al., J. Immunol., (1980) 124:548-552; Spits, et al.,  
25 Immunogenetics, (1982) 16:503-512; Gaston, et al., J. Exp. Med., (1983) 158:280-293).

Mutants which affect recognition by CTL have been studied in mice (Nathenson et al., Ann. Rev. Immunol. (1986) 4:471-502; Schulz et al., Proc. Natl. Acad. Sci. USA (1983, 80:207-2011) and humans, (Krangel Biochemistry (1982) 21:6313-6321; Krangel et al., J. Immunol. (1983) 130:1856-1862; Cowan et al., J. Immunol. (1985) 135:2835-2841; Taketani et al., ibid (1984)

133:816-821; and Vega et al., Proc. Natl. Acad. Sci. USA (1985) 82:7394-7398).

These reports have focused considerable  
5 attention on the region between residues 147 and 157,  
although other regions can also produce functional  
differences (Ezquerro et al., J. Immunol. (1985)  
134:2727-2733). Clusters of variability have been  
10 reported at the carboxy-terminal end of the first  
extracellular domain and at the amino-terminal end of  
the second extracellular domain (Ways et al., J. Biol.  
Chem. (1985) 26:11924-11933). Sequences between  
residues 105-108 of all Class I molecules are related to  
that of the fibronectin binding tetrapeptide (Auffray  
15 and Novotny, J. Human Immunology (1986) 15:381-390),  
which tetrapeptide in either orientation is found to  
have cell attachment properties (Pierschbacher and  
Ruoslahti, Nature (1984) 309:30-33; Yamada and Kennedy,  
J. Cell Biol. (1985) 28:99-104). Substitution at  
20 position 107 affecting a single monoclonal antibody  
defined epitope of HLA-A2 has been reported by Salter  
et al., J. Exp. Med. (1987) 166:283-288.

#### SUMMARY OF THE INVENTION

25 Methods and compositions are provided for  
modulating cytotoxic T-lymphocyte (CTL) activity toward  
target cells. The methods employ using peptide  
fragments cross-reactive with the region encompassing  
the  $\alpha_1$ - and  $\alpha_2$ -domains of major histocompatibility Class  
30 I antigens. Particularly, fragments which include at  
least a portion of the amino acids between positions 55  
and 120 of the Class I antigen are employed.

Accordingly, one aspect of the invention is a  
substantially pure preparation of peptide compound of at

least 8 amino acids and having a sequence coming within the extended sequence:

5                    aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> K  
                   aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup>  
                   aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A G S H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M aa<sup>99</sup> G  
                   C D aa<sup>103</sup> G aa<sup>105</sup> D aa<sup>107</sup> R aa<sup>109</sup> L R G aa<sup>113</sup> aa<sup>114</sup> Q  
                   aa<sup>116</sup> A Y D G

10

wherein:

                  aa<sup>55</sup> is E or K;  
                   aa<sup>62</sup> is G, Q, E or R;  
                   aa<sup>63</sup> is an acidic amino acid or amide thereof;  
 15                  aa<sup>65</sup> is Q, R or G;  
                   aa<sup>66</sup> is I, N or K;  
                   aa<sup>67</sup> is an aliphatic neutral amino acid;  
                   aa<sup>69</sup> is an aliphatic neutral amino acid;  
                   aa<sup>70</sup> is Q, H, S, N or K;  
 20                  aa<sup>71</sup> is an aliphatic neutral amino acid;  
                   aa<sup>74</sup> is D, Y or H;  
                   aa<sup>76</sup> is E or V;  
                   aa<sup>77</sup> is D, S or N;  
                   aa<sup>79</sup> is R or G;  
 25                  aa<sup>80</sup> is T, I or N;  
                   aa<sup>81</sup> is an aliphatic non-polar amino acid;  
                   aa<sup>82</sup> is R or L;  
                   aa<sup>83</sup> is G or R;  
                   aa<sup>94</sup> is T or I;  
 30                  aa<sup>95</sup> is a non-polar aliphatic amino acid of  
                   from 5 to 6 carbon atoms;  
                   aa<sup>97</sup> is an aliphatic amino acid or W;  
                   aa<sup>99</sup> is an aromatic amino acid;



aa<sup>103</sup> is a non-polar aliphatic amino acid of  
 from 5 to 6 carbon atoms;  
 aa<sup>105</sup> is P or S;  
 5 aa<sup>107</sup> is G or W;  
 aa<sup>109</sup> is L or F;  
 aa<sup>113</sup> is Y or H;  
 aa<sup>114</sup> is H, Q, D, N or R;  
 aa<sup>116</sup> is Y, D, S, F or H,  
 10 which modulates CTL activity.

Another aspect of the invention is a method of  
 modulating cytolytic activity of a CTL, said method  
 comprising:  
 15 combining CTL with a peptide compound of at  
 least 8 amino acids and not more than about 30 amino  
 acids having a sequence differing by not more than two  
 mutations from the polymorphic region of the C-terminal  
 half of the a<sub>1</sub> domain and the N-terminal half of the a<sub>2</sub>  
 20 domain of the host of said MHC restricted cells,  
 wherein said CTL cells are modulated by said  
 peptide.

Still another aspect of the invention is a  
 method for inhibiting cytolytic activity of a CTL, said  
 25 method comprising combining CTL with a peptide compound  
 of at least 8 amino acids and having a sequence coming  
 within the extended sequence:

30 G S H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M aa<sup>99</sup> G C D aa<sup>103</sup> G  
 aa<sup>105</sup> D aa<sup>107</sup> R aa<sup>109</sup> L R G aa<sup>113</sup> aa<sup>114</sup> Q aa<sup>116</sup> A Y D G

wherein:

aa<sup>94</sup> is T or I;

aa<sup>95</sup> is a non-polar aliphatic amino acid of  
from 5 to 6 carbon atoms;

aa<sup>97</sup> is an aliphatic amino acid or W;

5 aa<sup>99</sup> is an aromatic amino acid;

aa<sup>103</sup> is a non-polar aliphatic amino acid of  
from 5 to 6 carbon atoms;

aa<sup>105</sup> is P or S;

aa<sup>107</sup> is G or W;

10 aa<sup>109</sup> is L or F;

aa<sup>113</sup> is Y or H;

aa<sup>114</sup> is H, Q, D, N or R;

aa<sup>116</sup> is Y, D, S, F or H;

wherein said CTL cells are inhibited by said peptide.

15 Yet another aspect of the invention is a  
method of inhibiting cytolytic activity of a CTL, said  
method comprising combining CTL with a peptide compound  
of at least 8 amino acids and having a sequence coming  
within the extended sequence:

20

aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> K  
aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup>  
aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A G S H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M aa<sup>99</sup> G  
C D aa<sup>103</sup> G aa<sup>105</sup> D aa<sup>107</sup> R aa<sup>109</sup> LRG aa<sup>113</sup> aa<sup>114</sup> Q  
25 aa<sup>116</sup> A Y D G

wherein:

30 aa<sup>55</sup> is E or K;

aa<sup>62</sup> is G, Q, E or R;

aa<sup>63</sup> is an acidic amino acid or amide thereof;

aa<sup>65</sup> is Q, R or G;

aa<sup>66</sup> is I, N or K;

aa<sup>67</sup> is an aliphatic neutral amino acid;

- 5 aa<sup>69</sup> is an aliphatic neutral amino acid;  
 aa<sup>70</sup> is Q, H, S, N or K;  
 aa<sup>71</sup> is an aliphatic neutral amino acid;  
 aa<sup>74</sup> is D, Y or H;  
 aa<sup>76</sup> is E or V;  
 aa<sup>77</sup> is D, S or N;  
 aa<sup>79</sup> is R or G;  
 10 aa<sup>80</sup> is T, I or N, particularly T or I;  
 aa<sup>81</sup> is an aliphatic no-polar amino acid;  
 aa<sup>82</sup> is R or L, particularly R;  
 aa<sup>83</sup> is G or R;  
 aa<sup>94</sup> is T or I;  
 15 aa<sup>95</sup> is a non-polar aliphatic amino acid of  
 from 5 to 6 carbon atoms;  
 aa<sup>97</sup> is an aliphatic amino acid or W;  
 aa<sup>99</sup> is an aromatic amino acid;  
 aa<sup>103</sup> is a non-polar aliphatic amino acid of  
 from 5 to 6 carbon atoms;  
 20 aa<sup>105</sup> is P or S;  
 aa<sup>107</sup> is G or W;  
 aa<sup>109</sup> is L or F;  
 aa<sup>113</sup> is Y or H;  
 aa<sup>114</sup> is H, Q, D, N, or R;  
 25 aa<sup>116</sup> is Y, D, S, F, or H.

Another aspect of the invention is a method of  
 sensitizing MHC restricted cells to CTL, said method  
 comprising combining MHC restricted cells with a peptide  
 compound of at least 8 amino acids and having a sequence  
 30 coming within the extended sequence:

aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup>  
 aa<sup>66</sup> aa<sup>67</sup> K aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup>  
 aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A

aa<sup>55</sup> is E or K, particularly E;  
 aa<sup>62</sup> is G, Q, E or R, particularly R or G;  
 5 aa<sup>63</sup> is an acidic amino acid or amide thereof;  
 including E and N, particularly E;  
 aa<sup>65</sup> is Q, R or G, particularly Q or R;  
 aa<sup>66</sup> is I, N or K, particularly N or K;  
 aa<sup>67</sup> is an aliphatic neutral amino acid  
 10 including, V, M, S, C and Y, particularly V,  
 aa<sup>69</sup> is an aliphatic neutral amino acid  
 including, A, T and P, particularly A;  
 aa<sup>70</sup> is Q, H, S, N or K, particularly Q or H;  
 aa<sup>71</sup> is an aliphatic neutral amino acid  
 15 including S, A and T, particularly S;  
 aa<sup>74</sup> is D, Y or H, particularly D or H;  
 aa<sup>76</sup> is E or V;  
 aa<sup>77</sup> is D, S or N, particularly D;  
 aa<sup>79</sup> is R or G, particularly G;  
 20 aa<sup>80</sup> is T, I or N, particularly T or I;  
 aa<sup>81</sup> is an aliphatic non-polar amino acid  
 including L or A, particularly L;  
 aa<sup>82</sup> is R or L, particularly R;  
 aa<sup>83</sup> is G or R, particularly G.

25

Of particular interest is a sequence or sequence fragment of at least 8 amino acids of the sequence:

30

Q E G P E Y W D (G or R) (E or N) T (R or Q)  
 (K or N) V K A (H or Q) S Q T (H or D) R (V or E) (D,  
 S or N) L (G or R) (T or I) (L or A) (R or L) (G or  
 R) Y Y N Q S E A

wherein the CTL cells cause cytolysis of the MHC restricted cells combined with said peptide.

5 Still another aspect of the invention is a method for inhibiting cytolytic activity of a CTL by irreversibly inhibiting the CTL, said method comprising:  
combining a CTL with a peptide compound of at least 8 amino acids and having a sequence coming within  
10 the extended sequences:

T L Q R M Y G C D V G S D W R F L R G,

M Y G C D V G S D W R F L R G Y,

15 M Y G C D V G S D G R F L R G Y,

G P E Y W D G E T R K V K A, and

G P E Y W D R N T R N V K A,

20 wherein the peptide compound binds to the CTL.

Yet another aspect of the invention is a method for determining the presence of MHC restricted CTL's, said method comprising:

25 contacting cells with a peptide compound of at least 8 amino acids and having a sequence coming within the extended sequence:

30 aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> K  
aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup>  
aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A G S H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M aa<sup>99</sup> G  
C D aa<sup>103</sup> G aa<sup>105</sup> D aa<sup>107</sup> R aa<sup>109</sup> LRG aa<sup>113</sup> aa<sup>114</sup> Q  
aa<sup>116</sup> A Y D G

wherein:

- 5           aa<sup>55</sup> is E or K;  
          aa<sup>62</sup> is G, Q, E or R;  
          aa<sup>63</sup> is an acidic amino acid or amide thereof;  
          aa<sup>65</sup> is Q, R or G;  
          aa<sup>66</sup> is I, N or K;  
          aa<sup>67</sup> is an aliphatic neutral amino acid;  
          aa<sup>69</sup> is an aliphatic neutral amino acid;  
          aa<sup>70</sup> is Q, H, S, N or K;  
10          aa<sup>71</sup> is an aliphatic neutral amino acid;  
          aa<sup>74</sup> is D, Y or H;  
          aa<sup>76</sup> is E or V;  
          aa<sup>77</sup> is D, S or N;  
          aa<sup>79</sup> is R or G;  
15          aa<sup>80</sup> is T, I or N, particularly T or I;  
          aa<sup>81</sup> is an aliphatic no-polar amino acid;  
          aa<sup>82</sup> is R or L, particularly R;  
          aa<sup>83</sup> is G or R;  
          aa<sup>94</sup> is T or I;  
20          aa<sup>95</sup> is a non-polar aliphatic amino acid of  
          from 5 to 6 carbon atoms;  
          aa<sup>97</sup> is an aliphatic amino acid or W;  
          aa<sup>99</sup> is an aromatic amino acid;  
          aa<sup>103</sup> is a non-polar aliphatic amino acid of  
25          from 5 to 6 carbon atoms;  
          aa<sup>105</sup> is P or S;  
          aa<sup>107</sup> is G or W;  
          aa<sup>109</sup> is L or F;  
          aa<sup>113</sup> is Y or H;  
30          aa<sup>114</sup> is H, Q, D, N, or R;  
          aa<sup>116</sup> is Y, D, S, F, or H.

covalently joined to a compound capable of providing a detectable signal; and

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determining the presence of cells to which said detectable signal compound is specifically bound.

Still another aspect of the invention is a substantially pure preparation of peptide compound  
5 selected from the sequences:

10 T L Q R M Y G C D V G S D W R F L R G ,  
M Y G C D V G S D W R F L R G Y ,  
M Y G C D V G S D G R F L R G Y ,  
G P E Y W D G E T R K V K A , and  
15 G P E Y W D R N T R N V K A .

Yet another aspect of the invention is a pharmaceutical composition comprised of a peptide compound of at least 8 amino acids and having a sequence coming within the extended sequence,  
20

25 aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> K  
aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup>  
aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A G S H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M aa<sup>99</sup> G  
C D aa<sup>103</sup> G aa<sup>105</sup> D aa<sup>107</sup> R aa<sup>109</sup> L R G aa<sup>113</sup> aa<sup>114</sup> Q  
aa<sup>116</sup> A Y D G

wherein:

30 aa<sup>55</sup> is E or K;  
aa<sup>62</sup> is G, Q, E or R;  
aa<sup>63</sup> is an acidic amino acid or amide thereof;  
aa<sup>65</sup> is Q, R or G;  
aa<sup>66</sup> is I, N or K;  
aa<sup>67</sup> is an aliphatic neutral amino acid;  
aa<sup>69</sup> is an aliphatic neutral amino acid;

5           aa<sup>70</sup> is Q, H, S, N or K;  
          aa<sup>71</sup> is an aliphatic neutral amino acid;  
          aa<sup>74</sup> is D, Y or H;  
          aa<sup>76</sup> is E or V;  
          aa<sup>77</sup> is D, S or N;  
          aa<sup>79</sup> is R or G;  
          aa<sup>80</sup> is T, I or N;  
10           aa<sup>81</sup> is an aliphatic non-polar amino acid;  
          aa<sup>82</sup> is R or L;  
          aa<sup>83</sup> is G or R;  
          aa<sup>94</sup> is T or I;  
          aa<sup>95</sup> is a non-polar aliphatic amino acid of  
from 5 to 6 carbon atoms;  
15           aa<sup>97</sup> is an aliphatic amino acid or W;  
          aa<sup>99</sup> is an aromatic amino acid;  
          aa<sup>103</sup> is a non-polar aliphatic amino acid of  
from 5 to 6 carbon atoms;  
          aa<sup>105</sup> is P or S;  
20           aa<sup>107</sup> is G or W;  
          aa<sup>109</sup> is L or F;  
          aa<sup>113</sup> is Y or H;  
          aa<sup>114</sup> is H, Q, D, N or R;  
          aa<sup>116</sup> is Y, D, S, F or H.

25

          wherein said peptide compound is present in a  
pharmacologically effective dose in a pharmaceutically  
acceptable excipient.

30

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the minimum size of peptide  
sequence required for inhibition of cytolysis by HLA-A2  
specific CTL.



Fig. 2 shows the effect of pretreatment of CTL and of target cells on the inhibition of cytolysis by HLA-A2 specific CTL.

5 Fig. 3 shows the effect of peptide A2.98-113 on release of granules containing serine esterase during cytolysis of target cells by CTL.

10 Fig. 4 shows the consensus sequence of peptides which constitute the  $\alpha_1$ ,  $\alpha_2$ , and  $\alpha_3$  regions of a class I HLA molecule, as well as changes in these sequences in different specific HLA molecules.

Fig. 5 shows the effect of peptides from different HLA-A2 epitopes on cytolysis of target cells by CTL of different specificities.

15 Fig. 6 shows the sensitization of an HLA-Aw69 target cell to cytolysis by clone A2/B17 cells caused by peptide A2.56-69.

20 Fig. 7 shows the effect on sensitization of incubating target cells or clone A2/B17 cells with peptide A2.56-69.

#### DESCRIPTION OF THE SPECIFIC EMBODIMENTS

25 Methods and compositions are provided for modulating the effect of cytotoxic T lymphocytes or diagnosing the presence of CTL's of a predetermined specificity. The compositions are peptide sequences cross reactive with a polymorphic portion of the  $\alpha_1$  or  $\alpha_2$  domains of Class I major histocompatibility antigens, particularly the C-terminal portion of the  $\alpha_1$  domain and  
30 the N-terminal portion of the  $\alpha_2$  domain. These major histocompatibility antigens are classified as Groups A, B, and C, having a substantial number of subclassifications within these groups, where the different Class I antigens are able to bind specifically

to complementary CTL's, which are restricted by such antigens. Of particular interest for the  $\alpha_1$  domain is the amino acid sequence from positions 55 to 85, more particularly 55 to 80, usually 55 to 70, desirably including within the sequence a tetrapeptide DGET, GETR, DRET, or YWDG. Of particular interest for the  $\alpha_2$ -domain is the amino acid sequence from positions 90 to 120, more particularly 94 to 116, desirably including within the sequence a tetrapeptide SDWR or SDGR.

The peptides of interest which will serve as the receptor binding peptide will have at least 8 amino acids, usually at least 10 amino acids, and usually not more than about 30 amino acids, more usually not more than about 24 amino acids, desirably having about 12 to 21 amino acids. The amino acid sequence will usually not differ from a naturally occurring sequence by more than 2 amino acids, or mutations, e.g., deletions or insertions, more usually by not more than about 1 amino acid. The sequence employed will usually be from the polymorphic regions of the C-terminal half of the  $\alpha_1$  domain or the N-terminal half of the  $\alpha_2$  domain of the MHC antigen of the host of the MHC restricted T-cells, particularly an HLA-A group antigen.

For the most part, the amino acid sequences for the  $\alpha_1$  region will come within the following formula:

55 aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> K aa<sup>69</sup>  
 30 aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup>  
 aa<sup>83</sup> Y Y N Q S E A

aa<sup>55</sup> is E or K, particularly E;

aa<sup>62</sup> is G, Q, E or R, particularly R or G;

- aa<sup>63</sup> is an acidic amino acid or amide thereof;  
including E and N, particularly E;
- aa<sup>65</sup> is Q, R or G, particularly Q or R;
- 5 aa<sup>66</sup> is I, N or K, particularly N or K;
- aa<sup>67</sup> is an aliphatic neutral amino acid  
including, V, M, S, C and Y, particularly V,
- aa<sup>69</sup> is an aliphatic neutral amino acid  
including, A, T and P, particularly A;
- 10 aa<sup>70</sup> is Q, H, S, N or K, particularly Q or H;
- aa<sup>71</sup> is an aliphatic neutral amino acid  
including S, A and T, particularly S;
- aa<sup>74</sup> is D, Y or H, particularly D or H;
- aa<sup>76</sup> is E or V;
- 15 aa<sup>77</sup> is D, S or N, particularly D;
- aa<sup>79</sup> is R or G, particularly G;
- aa<sup>80</sup> is T, I or N, particularly T or I;
- aa<sup>81</sup> is an aliphatic non-polar amino acid  
including L or A, particularly L;
- 20 aa<sup>82</sup> is R or L, particularly R;
- aa<sup>83</sup> is G or R, particularly G.

The peptide may be extended by bonding at one  
or both termini with other than a wild-type sequence for  
25 the HLA-A2 antigen.

Of particular interest is a sequence or  
sequence fragment of at least 8 amino acids of the  
sequence:

30 Q E G P E Y W D (G or R) (E or N) T (R or Q) (K or N) V  
K A (H or Q) S Q T (H or D) R (V or E) (D, S or N) L (G  
or R) (T or I) (L or A) (R or L) (G or R) Y Y N Q S E A

For the most part, the amino acid sequences for the  $\alpha_2$  domain will come within the following formula:

5           91   100  
G S H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M aa<sup>99</sup> G C D aa<sup>103</sup> G aa<sup>105</sup> D  
.aa<sup>107</sup> R aa<sup>109</sup> L R G aa<sup>113</sup> aa<sup>114</sup> Q aa<sup>116</sup> A Y D G

10 aa<sup>94</sup> is T or I, particularly T;  
aa<sup>95</sup> is a non-polar aliphatic amino acid of  
from 5 to 6 carbon atoms, particularly I, L or V;  
aa<sup>97</sup> is an aliphatic amino acid, either polar  
or nonpolar, or W, including R, M, N, S, I, and W,  
particularly R;  
15 aa<sup>99</sup> is an aromatic amino acid, Y or F,  
particularly Y;  
aa<sup>103</sup> is a non-polar aliphatic amino acid of  
from 5 to 6 carbon atoms, particularly I, V, or L, more  
particularly V;  
20 aa<sup>105</sup> is P or S, particularly S;  
aa<sup>107</sup> is G or W, particularly W;  
aa<sup>109</sup> is L or F, particularly F;  
aa<sup>113</sup> is Y or H, particularly Y;  
aa<sup>114</sup> is H, Q, D, N or R, particularly H;  
25 aa<sup>116</sup> is Y, D, S, F or H, particularly Y or D.

The peptide may be extended by bonding at one or both termini with other than a wild-type sequence for the A2 antigen.

Of particular interest is a sequence or sequence fragment of at least 8 amino acids of the sequence:

G S H T [V, I, or L] Q R M Y G C D V G S D [W or G] R F  
L R G Y H Q Y A Y D G.

5           Where there are two or more amino acids  
indicated at the same site, any of the indicated amino  
acids may be present.

          The region of particular interest will be the  
region from amino acid positions 100 to 116.

10           The classes of amino acids are designated as  
follows:

          aliphatic

              non-polar G, A, P, L, I, V

              polar

15                 neutral C, S, T, M, N, Q

              acidic D, E

              basic K, R

          aromatic F, H, W, Y

20           The peptides may be prepared in a variety of  
ways. Conveniently, they can be synthesized by  
conventional techniques employing automatic  
synthesizers, such as the Beckman, Applied Biosystem  
Inc., or other useful peptide synthesizer apparatus, or  
may be synthesized manually. Alternatively, DNA  
25           sequences can be prepared which encode the particular  
peptide and may be cloned and expressed to provide the  
desired peptide. In this instance a methionine may be  
the first amino acid.

30           The peptides may also be isolated from natural  
sources and purified by known techniques, including, for  
example, chromatography on ion exchange materials,  
separation by size, immunoaffinity chromatography and  
electrophoresis. As used herein, the term "a  
substantially pure preparation of peptide compound"

means a preparation of the peptide which is usually greater than about 70% free of materials with which the polypeptide is naturally associated, and preferably  
5 greater than about 80% free of these materials; these materials, however, excludes materials with which the peptide may be mixed in the preparation of pharmaceutical compositions.

The sequences may be modified in a variety of  
10 ways depending upon their ultimate purpose. Different N- or C- terminal groups may be introduced which allow for linking of the peptide to solid substrates or other molecules. In a synthetic procedure, any molecule may be introduced at the N- or C-terminus which would allow  
15 for subsequent reaction, depending upon the purpose for which the peptide is prepared.

For diagnostic purposes, a wide variety of labels may be linked to the terminus, which may provide, directly or indirectly, a detectable signal. For  
20 example, fluorescers may be introduced at the terminus or other molecules which provide a linkage to labels such as fluorescers, enzymes, particles, or the like. For example, linkage may be introduced at the terminus, e.g., biotin, which will bind to an avidin conjugate  
25 with enzymes or fluorescers. Alternatively, various reactive sites may be introduced at the terminus for linking to particles, solid substrates, macromolecules, or the like. For example, an internal amino moiety of a growing chain bound to a solid substrate with the  
30 intermediate side groups protected, may be conjugated with methyldithiobenzoic acid (MDTB). The free mercaptan group may then be used for conjugating with activated olefins. Thus, proteins, such as serum albumin, keyhole limpet hemocyanin, bovine  $\beta$ -globulin,

or the like, may be conjugated to the peptide to provide for an immunogen to produce antibodies to the peptide for use in immunoassays, for affinity chromatography, or the like. Alternatively, the peptide can be bonded to another polypeptide by preparing a DNA sequence which has the peptide at the N-terminus, C-terminus or internal to the protein, so as to provide a fused protein which includes the binding peptide of interest. In this manner, fused proteins may be produced which have enzymatic activity, which enzymatic activity may be modulated by macromolecules, e.g., antibodies, binding to the peptide of interest. Thus, the peptides of the subject invention may be modified in a wide variety of ways for a variety of end purposes while still retaining biological activity.

The subject peptides may also be used in combination with antigenic peptides or proteins of interest to activate CTL's. Thus, the subject peptides may be bound to a protein, either directly or indirectly, so as to be able to present two epitopes to the CTL to which the CTL may bind and be activated. Of particular interest, is where the subject peptides may be bound to a liposome or a bilayer lipid membrane in conjunction with a peptide or protein providing the other determinant site.

Various techniques are available for joining a peptide or protein to a lipid, particularly a phospholipid to provide for the presence of the peptide or protein on the liposome surface. Phosphatidyl choline, phosphatidyl ethanolamine, or other lipid may be used with a bifunctional linking agent, such as MBSE, glutaraldehyde, methyldithiobenzoic acid, or the like. The formation of liposomes with conjugated proteins

finds ample support in the literature, see, for example, U.S. Patent Nos. 3,887,698; 4,261,975; and 4,193,983.

5 The modified peptide or protein is combined with the lipids in an aqueous medium and sonicated to provide the desired liposomes. The liposomes may then be harvested and used in the ways indicated.

10 The subject peptides, by themselves, or in combination with other peptides or proteins, may be used for diagnosing the presence of CTL's which bind to a subject peptide or the combination of a subject peptide and other peptide or protein. In this manner, conjugates of the subject peptide and the antigenic peptide or protein can be prepared by employing linking  
15 agents as described previously. Alternatively, the subject peptide and the antigenic peptide may be bound to a solid surface, such as a particle, container surface, or the like. If desired, the subject peptide and antigenic peptide or protein may be conjugated to a  
20 particle or protein which is fluorescent. The binding of the particle or protein will allow for sorting and counting in a fluorescence activated cell sorter.

The subject peptides may also be used for modulating CTL activity in the mammalian host. The  
25 modulation may be by inhibiting CTL activity or by sensitizing target cells. This can be achieved by employing apheresis, where the patient's blood is withdrawn from the patient and circulated through a device in which the peptide is present, either bound to  
30 the surface, to remove CTL's active with the subject peptide or in a physiologically acceptable medium to bind to the CTL's and inhibit their activity. Alternatively, the subject peptides may be administered



to a host intravascularly, in either an artery or vein, to provide for inhibition or stimulation of the CTL.

5        Examples of inhibitory peptides are presented  
infra (see Examples 2 and 9), which are derived from  
both the  $\alpha_1$  and  $\alpha_2$  domain of HLA-A2. In each case the  
sequence of the inhibitory peptide correlates with the  
epitope specificity of the CTL. Moreover, as shown in  
10    Example 4, inhibition is mediated by an octapeptide, and  
occurs by peptide binding to the CTL and not the target  
cell (see Example 5). Since the inhibitory capacity of  
the individual peptides correlates with CTL specificity,  
it seems likely that these peptides inhibit by binding  
to the variable T cell receptor.

15        An example of a peptide which stimulates  
cytolysis of a HLA-class I bearing target cells by  
alloreactive CTL is presented in Example 10, infra. The  
simplest interpretation of the results in Examples 10-12  
is that the HLA-A2/B17 specific CTL recognize the A2  
20    56-69 peptide in the context of HLA-Aw69 as a  
restriction element. This implies that the peptide is  
binding to the HLA-Aw69 molecule. The data and  
interpretation are similar to those obtained in the  
influenza (Bastin et al., J. Exp. Med., 165:1508 (1987);  
25    Gotch et al., Nature 326:881 (1987)) and xenogeneic  
systems (Maryanski et al., Nature 324:578 (1986)), and  
demonstrate that alloreactive CTL can recognize Class I  
derived peptides in a Class I restricted fashion.  
However, the quantities of peptide required to cause  
30    sensitization are significantly larger than those  
reported in other studies. Although the molecular basis  
for this is as yet unknown, one possible explanation  
involves the relative handling of exogenous versus  
endogenous molecules. For example, HLA is an endogenous

molecule, and the exogenous HLA peptides may have to compete with endogenous HLA peptides. Another alternative is that the A2.56-69 peptide may not include  
5 all of the residues required for high affinity binding to the target cell.

Two of the CTL epitopes from which the peptides described in the Examples, *infra.* are derived, are situated in very different parts of the HLA-A2  
10 molecule. Residues 62-65 are in an alpha helix which forms part of the peptide binding site (Bjorkman et al., Nature 329:506 (1987)), which is itself thought capable of binding alpha helical peptides. As shown in the  
15 Examples, peptides in this region can either inhibit or induce cytolysis. In the induction of cytolysis, it is possible that the peptide may bind to a target cell HLA class I antigen and thereby create a structure which is recognized by the CTL. For example, in the case of  
20 A2.56-69 peptide conferring sensitivity to clone A2/B17 cells on HLA-Aw69 cells, the bound peptide presumably substitutes for the  $\alpha$  helix of the  $\alpha_1$  domain, since HLA-Aw69 and HLA-A2 have identical  $\alpha_2$  domains.

In contrast, residue 107 is part of a turn between two strands of  $\beta$  structure at some distance from  
25 the alpha helices and peptide binding region (Bjorkman et al., supra.) The A2.98-113 peptide may maintain elements of this structure in solution and have little affinity for the peptide binding site of Class I molecules. This interpretation would explain the  
30 observation in the Examples that peptides corresponding to this region are inhibitors of HLA-A2 directed cytolysis, but cannot induce cytolysis.

As already indicated, the peptide may be present by itself, or in combination with an antigen

thereby providing a different determinant site of interest. Depending upon whether only the subject peptide is included, or the peptide in combination with other peptides, activation or inhibition can be achieved.

If irreversible inhibition is desired, the conjugate of the subject peptide with the antigen may be joined to a cytotoxic agent, joined to liposomes containing cytotoxic agents, or joined to a specific monoclonal antibody or immunoglobulin, whereby binding of the conjugate to the CTL will result in the complement mediated lysis of the CTL.

The subject peptides, by themselves or as conjugates, may be prepared as formulations in pharmaceutically acceptable media, for example saline, PBS, and glucose, generally at a pharmacologically effective dose, the concentrations of which will be determined empirically in accordance with conventional procedures for the particular purpose. The additives may include bactericidal agents, stabilizers, buffers, or the like. The amount administered to the host will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, whether inhibition or activation is desired, the state of the host, the manner of administration, and the like. In order to enhance the half-life of the subject peptide or subject peptide conjugates, the peptides may be encapsulated, introduced into the lumen of liposomes, prepared as a colloid, or other conventional technique may be employed which provides an extended lifetime of the peptides.

The following examples are offered by way of illustration and not by limitation.

EXAMPLES

5

Example 1Preparation of Peptides Derived From  
HLA-A2

Four peptides were prepared by conventional synthetic methods using standard solid-phase methods.  
10 See Erickson & Merrifield in: The Proteins Vol. 2, 3rd edition (eds. Neurath, H. & Hill, R.L.) p. 255-527 (Academic Press, N.Y. 1970), which is hereby incorporated herein by reference. Three of the peptides had amino acids from the  $\alpha_2$  domain and one of the  
15 peptides had amino acids from the  $\alpha_2$  domain of a HLA-A2 antigen. The four peptides had the following compositions and designations:

A2.56-69 G P E Y W D G E T R K V K A  
20 A2.94-112 T L Q R M Y G C D V G S D W R F L R G  
A2.98-113 M Y G C D V G S D W R F L R G Y  
25 Aw.68 98-113 M Y G C D V G S D G R F L R G Y

The designations indicate the major histocompatibility antigen from which the peptide is derived, and the position of the amino acids in the  
30 antigen.

Example 2  
Inhibition of HLA-A2 Specific CTL  
by Peptides Derived from HLA-A2.98-113  
and HLA-A2.94-112

5           Peptides prepared as in Example 1, i.e., those  
corresponding to HLA-A2.56-69, HLA-A2.94-112,  
HLA-A2.98-113, and HLA-Aw 68.98-113, were preincubated  
for 30 min. with  $1-3 \times 10^3$  CTLs before addition of  $10^3$   
10 CPM of  $^{51}\text{Cr}$ -labeled B-lymphoblastoid target cells. The  
cytotoxicity assay was then performed as described by  
Clayberger et al., J. Exp. Med. (1984) 162:1709-1714;  
and Reiss et al., Proc. Natl. Acad. Sci. USA (1980)  
77:5432-5436, which are hereby incorporated herein by  
15 reference.

          In the first study, the CTL cell line was AJY,  
a long term  $\text{CD8}^+$  CTL line specific for HLA-A2, and the  
target cell was the B-lymphoblastoid cell line JY  
(HLA-A2, B7). In the second study the CTL was PWSB, a  
20 bulk culture with reactivity against HLA-B17 and the  
target was FMB, which expresses HLA-A1, A32, B17. In  
each case the percentage of specific release obtained in  
the absence of peptide was determined. The lower amount  
of specific release in the second study potentially made  
25 cytotoxicity more sensitive to inhibition. Stocks of  
peptides at 1 mg/ml in PBS were diluted to give final  
concentrations in the assay as indicated in Table 1. As  
a control inhibitor, the monoclonal antibody PA2.6 which  
is directed against the monomorphic determinant of  
30 HLA-A, B, C molecules was used (Reiss et al., supra:  
McMichael, J. Exp. Med. (1980) 152:195s-203s). The  
peptides employed were A2.98-113, A2.94-112, Aw68. 94  
112 and A2.56-69. The following table indicates the  
results.

Table 1

		<u>% Specific Lysis</u>				
		Concentration	A2.98-113	A2.94-112	Aw68.94-112	A2.56-69
		<u>uq/ml</u>				
5	Trial 1.	160	0	3	52	51
	CTL=AJY	80	4	20	45	38
	Target=JY	40	18	35	63	61
	Trial 2.	160	27	35	28	20
	CTL=PWSB	80	29	32	30	27
	Target=FMB	40	30	34	35	31
10						

15 In the first case, the percentage specific release obtained in the absence of peptide was about 54, while in the second case it was about 28.

The above results with CTL which are restricted by the HLA-A2 antigen, show inhibition of specific cytotoxicity. With CTL's not restricted by A2, lysis of random target cells occurs with the results approximating the standard specific release obtained in the absence of peptide. These results suggest that the tryptophan at position 107 may be critical. Peptide  
20 A2.98-113 and peptide Aw68.98-113 are homologous except for the substitution of glycine for tryptophan at this position; this substitution resulted in a loss of inhibition of cytolysis by HLA-A2 specific CTL.

30 The results of treatment of peptide A2.98-113 with different proteases, i.e., trypsin or chymotrypsin, allow the suggestion that arginine 108 is of importance, but that peptides 109-113 are not critical. The major sites of action of trypsin and chymotrypsin are Arg, Lys, and Trp, Phe, Tyr, respectively. Chymotryptic, but

not tryptic, cleavage of the peptide reduced the inhibitory activity (results not shown).

Example 3

Effect of Specificity of CTL and Target Cell  
on Inhibition of Cytolysis Caused by  
HLA-Derived Peptides

A number of different CTL cell lines were studied, where the specificity of the cell lines were varied. The results shown in Table 2 indicate that only where the CTL's and the target cells share A2 specificity do the A2-derived peptides provide inhibition.

Table 2  
Specificity of CTL Tested for Inhibition by Peptides

CTL	Specificity of CTL	Target Cell	Target Molecule	Inhibition of Lysis by Peptide		
				A2.98-113	A2.94-112	A2.56-69
Line AJY	A2	JY	A2	+	+	-
Line PJY	A2	JY	A2	+	+	-
Clone A20.1	A2	JY	A2	+	+	-
Clone AI.10	A2	JY	A2	+	+	-
Clone AI9.1	A2, Aw68, Aw69	JY	A2	+	+	-
Line PWSB	A2, B17	JY	A2	+	+	-
Line PWSB	A2, B17	FMB	B17	-	-	-
Clone AL8.1	Aw68, Aw69	LB	Aw68	-	-	ND
Clone AI5.1	Aw69	IDF	Aw69	-	-	ND
Line CJY	Dr6	JY	DR6	-	ND	-
Line CJY	Dr6	DAUDI	DR6	-	ND	-

The specificity of the CTL is based upon analysis of the pattern of killing on a panel of B lymphoblastoid cell lines and by patterns of inhibition with monoclonal antibodies. ND indicates not done. CTL used were from four different donors.



Example 4Minimum Peptide Sequence Required  
for Inhibition of HLA-A2 Specific CTL

5           The minimum peptide sequence required for inhibition of cytolysis by HLA-A2 specific CTL was determined by examining the effect of size on the inhibition.

10           A series of peptides which started at positions 98-104 and ended at position 108 of HLA-A2 or HLA-Aw68 were synthesized. The effect of these peptides on cytolysis of JY cells (HLA-A2, B7, DR4,6) by seventeen different HLA-A specific lines or clones were tested. The HLA-A2 specific lines or clones were  
15           generated as described in Clayberger et al., supra. Peptides (200 mg/ml) were preincubated with  $1-3 \times 10^3$  CTL for 30 minutes prior to addition of  $10^3$  CPM of  $^{51}\text{Cr}$ -labeled target cells. The peptides were present throughout the cytotoxicity assay which was performed as  
20           described in Clayberger et al., supra, and in Krensky et al., Proc. Natl. Acad. Sci. USA 79:2365 (1982), which are hereby incorporated herein by reference. Peptides were prepared as stock solutions at 1 mg/ml in phosphate buffered saline and diluted in complete medium (MEM  
25           supplemented with 10% calf serum) to give the final concentration used.

30           The results on the inhibition of cytolysis by CTL-A2 is shown in Fig. 1A, where inhibition is expressed as  $(1 - [\text{specific cytolysis in the presence of peptide} / \text{specific cytolysis in the absence of peptide}]) \times 100$ .

As seen in the figure, peptide 104-108 did not inhibit, peptides 102-108 and 103-108 caused weak inhibition, and the remaining peptides caused good

inhibition of cytolysis. Thus, an octapeptide comprising residues 101-108 was sufficient to cause the inhibitory effect.

5           A major decrease in the inhibitory effect occurs with loss of the cysteine at position 101. This loss may be due to the loss of disulfide cross-linking of two peptide molecules when cysteine 101 is absent.

10                           Example 5

Locus of Action of Peptide A2.98-113

          The locus at which peptide A2.98-113 interacts to cause an inhibitory effect on HLA-A2 specific CTL mediated cytolysis, i.e., with the CTL and/or with the target cell, was determined as follows.

15           The CTL ( $1 \times 10^6$  CTL-A2) and/or the target cells ( $^{51}\text{Cr}$ -labeled JY target cells) were incubated with 100  $\mu\text{g}$  of A2.98-113 for 30 min. at  $37^\circ\text{C}$ , or alternatively with the control peptide, Aw68.98-113.

20           The sequences of these peptides are presented in Example 1. As an additional control, the cells were incubated with complete medium minus peptide. Following the incubation, the cells were washed three times in complete medium, and tested in a  $^{51}\text{Cr}$ -release assay (see

25           Example 2).

          The results are presented in Fig. 2, where it may be seen that lysis was inhibited when the CTL, but not the target cells, were pretreated with A2.98-113. Inhibitory effects were not observed when CTL or target

30           cells were pretreated with the control peptide, Aw68.98-113.

Example 6Mechanism of Inhibition of CTL by A2.98-113Effect on CTL Viability

5           To determine whether CTL were inhibited due to  
their autolysis induced by A2.98-113, either  
51Cr-labeled CTL-A2 cells or unlabeled CTL-A2 cells were  
incubated with the peptide for 6 hours at 37°C in  
complete medium. During the 6 hour incubation there was  
10 no detectable decrease in cell viability as judged by  
exclusion of trypan blue or by 51Cr-release (results not  
shown).

Example 7

15           Mechanism of Inhibition of CTL by A2.98-113

Effect on Release of Granules ContainingSerine Esterase

20           The effect of A2.98-113 on release of granules  
containing serine esterase during cytolysis of target  
cells by CTL was determined as follows.

          The specificity of release was determined by  
incubating  $3 \times 10^5$  HLA-A2 specific CTL with JY cells  
(HLA-A2; B7; Dr4,6) or IBW4 cells (HLA-A3; B35; DR1) for  
2 hours in V bottom microtiter wells. The ratios of  
25 CTL:target cells were 1:0.01, 1:0.05, 1:0.10, 1:0.5, and  
1:1. After the incubation, the plates were spun at 1000  
RPM for 2 minutes, and the supernatant was assayed for  
serine esterase activity essentially as described in  
Young et al., Cell 47:183 (1986), which is hereby  
30 incorporated herein by reference. The reaction mixtures  
consisted of 20  $\mu$ l of supernatant plus 200  $\mu$ l of  
substrate ( $2 \times 10^{-4}$  M N-benzyloxycarbonyl-L-lysine  
thiobenzyl ester,  $2.2 \times 10^{-4}$  M nitrobenzoic acid, 0.1M  
Tris-HCl, pH 8.0). After 30 min. at 37°C, the

absorbance was determined at 410 nm. Total serine esterase activity was determined by substituting 0.01% Triton X-100 for stimulator cells.

5           The results, shown in Fig. 3A, indicate that release of the granules occurred when the HLA-A2 specific CTL were incubated with JY cells (closed circles), but not when the HLA-A2 specific CTL were incubated with IBW4 cells (closed squares).

10           The effect of peptide A2.98-113 on release of granules containing serine esterase was determined in a similar fashion, except that the HLA-A2 specific CTL were preincubated with 100 µg of peptide, either A2.98-113 or Aw68.98-113, or with only complete medium,  
15           for 30 min. at 37°C prior to the addition of JY target cells at ratios of CTL:target cells of 1:0.01, 1:0.05, 1:0.1, 1:0.5 and 1:1.

          As seen in Fig. 3B, complete inhibition of esterase release was seen with 100 µg/ml of A2.98-113 at  
20           an effector-to-target ratio of 1:0.1 (closed squares). The control peptide Aw68.98-113 had no effect on esterase release (closed triangles), since release in this case was equal to that obtained with control cells preincubated with complete medium (closed circles).

25           These results, in conjunction with those in Example 5 indicate that the A2.98-113 peptide blocks events which occur early in T cell activation by binding directly to the CTL. This binding may be to the antigen receptor.

30

Example 8Isolation of CTL Specific for the Epitope Shared  
by HLA-A2 and HLA-B17, for HLA-B17, and for HLA-2

5 CTL with the various specificities were  
derived from the peripheral blood lymphocytes of a  
normal donor (HLA-A3; B7; DR6) essentially as described  
by Clayberger et al. (1985), supra. For CTL specific  
for the epitope shared between HLA-A2 and HLA-B17, the  
10 cells were stimulated in primary culture with the  
irradiated (10,000R) B-lymphoblastoid cell line Mag  
(HLA-A26,33; B17,51) and cloned using the SB cell line  
(HLA-A1,2; B17,44; DR2,6) as stimulators. CTL specific  
for B17 were derived from the same primary culture, but  
15 were cloned using the SH cell line (HLA-A3,w33;  
B7,17(w57)) as stimulators. HLA-A2 specific CTL were  
derived from cell stimulated in primary culture with the  
JY cell line and cloned using the Herluff cell line  
(HLA-A2; B12,35; DR4,7) as stimulators. The fine  
20 specificity of these CTL clones was assessed using a  
panel of 11 targets expressing HLA-B17, 8 targets  
expressing HLA-A2 and 15 targets with unrelated HLA  
molecules. Multiple clones of the desired specificities  
were obtained. An individual clone which caused  
25 cytolysis of both HLA-A2 type target cells and HLA-B17  
type target cells was designated clone A2/B17. The  
cytolysis of target cells of clone A2/B17 was inhibited  
by antibody MA2.1. A second clone, which lysed all  
HLA-B17 target cells but no others was designated B17.  
30 A third clone, which lysed all HLA-A2 target cells but  
no others was designated CTL-A2.

The target specificity of clone A2/B17 and the  
finding that cytolysis by this clone was blocked by  
monoclonal antibody MA2.1 indicates that cells of clone

A2/B17 recognize the epitope shared by HLA-A2 and HLA-B17.

Example 9

The Effect of Peptides from Different HLA-A2 Epitopes  
on Cytolysis of Target Cells by CTL  
of Different Specificities

5  
10 Examples 2-7, supra, have involved the effects of peptides derived from the region around tryptophan 107 in the  $\alpha_2$  domain. This residue, which is on a bend between two strands of  $\beta$  pleated sheet (Bjorkman et al., (1987), supra), is critical for a major serologic epitope of HLA-A2. Salter et al., J. Exp. Med. 166:283 (1987); Layet et al., J. Immunol. 138:2197 (1987).

15 Another important epitope involves residues 62-65 of the  $\alpha$  helical region of the  $\alpha_1$  domain. Bjorkman et al., supra. This epitope was originally defined by the monoclonal antibody MA2.1 (McMichael et al., Hum. Immunol. 1:121 (1980)), and is shared by all  
20 known subtypes of HLA-A2 and HLA-B17 (Ways and Parham, Biochem. J. 216:423 (1983)). A comparison of the amino acid sequence of HLA-A2 and HLA-B17 and eight other  
25 HLA-A,B,C proteins showed that only the glycine residue at position 62 is unique, suggesting that this residue contributes to a shared determinants (Ways et al., J. Immunol. 137:217 (1986)).

30 Peptides derived from the above two regions were examined for their inhibitory effect on cytolysis of target cells by CTL with different HLA specificities, i.e., those of clone A2/B17, clone CTL-A2, and clone B17 (see Example 8, supra). CTL were incubated with the following peptides: A2.56-69, Aw68.56-69, A2.98-113, or Aw68.98-113.

The epitopes studied and peptides used in the study are shown in Fig. 4, where the protein sequences in the three extracellular domains ( $\alpha_1$ ,  $\alpha_2$  and  $\alpha_3$ ) of eight HLA-A,B molecules are shown using the standard one letter amino acid code. The sequence of HLA.Bw58 subtype of HLA-B17 is from Ways *et al.*, *J. Biol. Chem.* 260:11924 (1985), that of HLA-A3.1 is from Strachen *et al.*, *EMBO J* 3:887 (1984), and the remaining sequences of the HLA-A2/28 family are from Holmes *et al.*, *J. Immunol.* 139:936 (1987). Peptides A2.56-69 and Aw68.56-69, and A2.98-113 and Aw68.98-113, which are derived from  $\alpha_1$  and  $\alpha_2$ , respectively, are indicated by cross-hatching. The two residues found to be critical for the epitopes shared by subtypes of HLA-A2 and HLA-B17 (glycine 62) and subtypes HLA-A2 and HLA-Aw69 (tryptophan 107) are indicated by stippling and the vertical arrows. The consensus sequence is derived from a total of 23 HLA-A,B,C sequences.

The CTL were incubated with peptides at concentrations of 100  $\mu\text{g/ml}$ , 200  $\mu\text{g/ml}$ , or 300  $\mu\text{g/ml}$ . Control samples were incubated in the absence of peptide. The final molar concentrations of peptides used in the assay at 100  $\mu\text{g/ml}$  were  $4.9 \times 10^{-5}\text{M}$  for A2.98-113;  $5.2 \times 10^{-5}\text{M}$  for Aw68.98-113;  $5.9 \times 10^{-5}\text{M}$  for A2.56-69; and  $5.9 \times 10^{-5}\text{M}$  for Aw68.56-69. The CTL cells were incubated with the peptides for 20 min. prior to the addition of  $10^3$   $^{51}\text{Cr}$ -labeled T7529 cells (HLA-Aw33; B17(w58); DR6) or JY cells (HLA-A2; B17; DR4,6). In all cases, the effector-to-target ratios were 1:1.

The results on cytotoxicity, as measured by  $^{51}\text{chromium}$  release from the target cells, is shown in Fig. 5. Figures 5A and 5B show the results of the effects of the peptides on cells of clone A2/B17; Fig.

5C shows the effects on cells of clone B17, and Fig. 5D on CTL-A2. The peptides are indicated as follows: (open circles) A2.56-69; (open squares) Aw68.56-69; (open triangles) Aw.98-113; and (closed squares) Aw68.98-113.

Peptide A2.56-69, which encompasses the shared serologic epitope, specifically inhibited the killing of both HLA-A2 and HLA-B17 expressing target cells by clone A2/B17 cells. In contrast, this peptide had no effect upon the lysis of HLA-B17 expressing cells by clone B17 cells. Clone A2/B17 cells were not inhibited by a peptide derived from residues 56-69 of HLA-Aw68.1, or by a series of unrelated peptides. The A2.98-113 peptide did not affect the lysis of HLA-B17 expressing targets by clone A2/B17 cells, but some inhibition was observed at high concentrations with HLA-A2 expressing targets. This difference indicates that the epitopes of HLA-A2 and HLA-B17 recognized by clone A2/B17 cells are not precisely the same.

These results show that the capacity of peptides to inhibit alloreactive CTL is not restricted to the region involving residues 101-108 of the  $\alpha_2$  domain, and that they may be derived from a second epitope of HLA-A2.

The discrepancy of the results achieved with peptide A2.56-69 using clone A2/B17, and those with the PWSB cell line (see Table 2) with respect to the inhibitory effect of this peptide may be explained by the polyclonal nature of the PWSB cells. That is, the PWSB line probably is a mixture of CTL's including individual clones specific for HLA-A2 or HLA-B17.



Example 10Sensitization of Target Cells to CTL caused by a  
HLA-2 Derived Polypeptide

5           Clone A2/B17 was incubated with peptide  
A2.56-69 and <sup>51</sup>Cr-labeled target cells at an  
effector-to-target ratio of 5:1 for 5 hours, after which  
<sup>51</sup>chromium released was measured. The concentrations of  
peptide were 10, 30, 100, and 300 µg/ml. The results of  
10 the effect of peptide on the percent of specific lysis  
of the target cells by clone A2/B17 cells are presented  
in Fig. 6. The target cells were: (closed square), IBW4  
(HLA-A3; B35; DR1); (closed triangle), LB (HLA-Aw68.1;  
B40, DR6); (closed circle), Pally (HLA-Aw68.2, 26;  
15 B14, 38; DR1, 4), or (open diamond), IDF (HLA-Aw69, 26;  
B18, 38, DR5).

In the absence of peptide, clone A2/B17 cells  
do not lyse targets expressing HLA-Aw69, HLA-Aw68.1, and  
HLA-Aw68.2 (data not shown). The inability of clone  
20 A2/B17 cells to lyse these targets is due to the  
differences in the critical residues around position 62  
from those found in HLA-A2 and HLA-B17. However, when  
peptide A2.56-69 was included in the cytotoxicity assay,  
there was significant lysis of HLA-Aw69 expressing  
25 targets by A2/B17 cells (Fig. 5). In contrast, targets  
expressing HLA-Aw68.1, HLA-Aw68.2, or the unrelated  
HLA-A3 molecule were not lysed.

Lysis of HLA-Aw69 cells by clone A2/B17 cells  
in the presence of peptide A2.56-69 was blocked by  
30 monoclonal antibody DR11-351, which only binds to the  
HLA-Aw69 of the target cell. In contrast, the  
monoclonal antibody MA2.1 did not inhibit lysis (results  
not shown). MA2.1 binds to the epitope of HLA-A2 and  
HLA-B17 formed by residues 56-69, but does not bind to

the HLA-Aw69 or peptide A2.56-69. These results demonstrate the involvement of the HLA-Aw69 molecule in the sensitization by peptide A2.56-69.

5           The addition of A2.98-113 peptide to B cell lines which do not express HLA-A2 did not cause sensitization to lysis when target cells expressing a variety of HLA molecules were used. This was true even though a wide range of peptide concentrations (0.1 to  
10   300 µg/ml were used.)

          In binding A2.56-69, the HLA-Aw69 molecule is able to present an epitope that mimics the native structure of HLA-A2. That HLA-Aw69 but not other members of the HLA-A2/28 family can be sensitized is of  
15   interest. HLA-Aw69 is a recombinant molecule having  $\alpha_1$  derived from HLA-Aw68 and  $\alpha_2$  and  $\alpha_3$  derived from HLA-A2.1 (Holmes and Parham, EMBO J. 4:2849 (1985)). Thus, HLA-2.1 and HLA-Aw69 differ by only 6 amino acids, all residing in the  $\alpha_1$  domain and three of which are  
20   present in the A2.56-69 peptide.

#### Example 11

##### Locus of Peptide Interaction in Sensitization

          To assess whether sensitization resulted from  
25   peptide interaction with the CTL or the target, cells were pretreated with A2.56-69, washed and then tested for cytolysis. More specifically,  $1 \times 10^6$  clone A2/B17 cells or  $^{51}\text{Cr}$ -labeled IDF (HLA-Aw69,26; B18,38; DR5) were incubated with 100 µg of peptide or medium for 30  
30   min. at 37°C, washed three times, and cytotoxicity as determined by  $^{51}\text{chromium}$  release was measured.

          As seen from the results presented in Fig. 7, target cells expressing HLA-Aw69 were lysed when the targets, but not the CTL, were pretreated with A2.56-69.

Example 12Effect of Peptide A2.56-69 on Release of  
Granules Containing Serine Esterase

5           The effect of peptide A2.56-69 on the release  
of granules containing serine esterase during co-culture  
of A2/B17 cells with HLA-Aw69 expressing cells may be  
essentially as described in Example 7, supra, except  
10   that the CTL are from clone A2/B17, the target cells are  
those expressing HLA-Aw69, and the cells are co-cultured  
in the absence or presence of peptide A2.56-69.

          Although the foregoing invention has been  
described in some detail by way of illustration and  
15   example for purposes of clarity of understanding, it  
will be obvious that certain changes and modifications  
may be practiced within the scope of the appended  
claims.

UTILITY

20           It is evident from the above results that  
cytotoxic cells can be inhibited employing the subject  
peptides which are cross-reactive with the polymorphic  
regions of a Class I MHC antigen, as demonstrated with  
25   the A2 antigen. In this way, CTL's may be specifically  
inhibited from lysing target cells that express the same  
antigen that CTL's are restricted by. This is of use in  
the transplantation of organic tissue to prevent host  
rejection of tissue which is restricted by CTL. In  
30   addition, it may be of use in prevention/therapy of  
autoimmune disease.

          Alternatively, the CTL's may be activated by  
employing specific conjugates involving the subject  
oligopeptides in conjunction with an antigen of

interest. This is useful in activating CTL to lyse cells carrying antigens other than those recognized by the CTL, and thus may induce CTL to lyse cells carrying  
5 antigens which are cryptic to the host, for example, in parasitic diseases and in neoplasia.

The subject compounds may also be used in viral studies in determining MHC sequences associated with restriction of T-lymphocytes in the case of viral  
10 infection.

The peptides of the invention may also be used to determine the presence or absence of CTL which are targeted towards cells bearing the HLA-Class I molecule from which the peptide is derived. Knowledge of the  
15 presence or absence of the targeted CTL is useful in predicting the success or failure and/or requirement for CTL modulation in transplantation of organic tissue. It may also be helpful in determining the mechanisms whereby cells become cryptic to the immune system. In  
20 addition these peptides may also be helpful in the detection of autoimmune diseases.

25

30

WHAT IS CLAIMED IS:

1. A substantially pure preparation of  
 5 peptide compound of at least 8 amino acids and having a  
 sequence coming within the extended sequence

aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> K  
 aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup>  
 10 aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A G S H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M aa<sup>99</sup> G  
 C D aa<sup>103</sup> G aa<sup>105</sup> D aa<sup>107</sup> R aa<sup>109</sup> L R G aa<sup>113</sup> aa<sup>114</sup> Q  
 aa<sup>116</sup> A Y D G

wherein:

- 15 aa<sup>55</sup> is E or K;  
 aa<sup>62</sup> is G, Q, E or R;  
 aa<sup>63</sup> is an acidic amino acid or amide thereof;  
 aa<sup>65</sup> is Q, R or G;  
 aa<sup>66</sup> is I, N or K;  
 20 aa<sup>67</sup> is an aliphatic neutral amino acid;  
 aa<sup>69</sup> is an aliphatic neutral amino acid;  
 aa<sup>70</sup> is Q, H, S, N or K;  
 aa<sup>71</sup> is an aliphatic neutral amino acid;  
 aa<sup>74</sup> is D, Y or H;  
 25 aa<sup>76</sup> is E or V;  
 aa<sup>77</sup> is D, S or N;  
 aa<sup>79</sup> is R or G;  
 aa<sup>80</sup> is T, I or N;  
 aa<sup>81</sup> is an aliphatic non-polar amino acid;  
 30 aa<sup>82</sup> is R or L;  
 aa<sup>83</sup> is G or R;  
 aa<sup>94</sup> is T or I;  
 aa<sup>95</sup> is a non-polar aliphatic amino acid of  
 from 5 to 6 carbon atoms;

aa<sup>97</sup> is an aliphatic amino acid or W;  
aa<sup>99</sup> is an aromatic amino acid;  
aa<sup>103</sup> is a non-polar aliphatic amino acid of  
5 from 5 to 6 carbon atoms;  
aa<sup>105</sup> is P or S;  
aa<sup>107</sup> is G or W;  
aa<sup>109</sup> is L or F;  
aa<sup>113</sup> is Y or H;  
10 aa<sup>114</sup> is H, Q, D, N or R;  
aa<sup>116</sup> is Y, D, S, F or H,  
which modulates CTL activity.

2. A peptide compound according to Claim 1,  
15 comprising at least eight amino acids within the  
sequence:

G S H T (Y or L) I Q R M Y G C D V G S D W G F  
L L R G Y H Q Y A Y D G,

20

or

Q E G P E Y W D (G or R) (E or N) T (R or Q)  
(K or N) V K A (H or Q) S Q T (H or D) R (V or  
25 E) (D, S or N) L (G or R) (T or I) (L or A) (R  
or L) (G or R) Y Y N Q S E A,

wherein the amino acids in the parentheses indicate that  
any of the amino acids in the parentheses may be  
30 present.

3. A peptide compound according to Claim 1,  
which is extended by bonding at least one terminus to  
other than a wild-type sequence of the natural human MHC

antigen having the same amino acid sequence at the N-terminus of the  $\alpha$ -domain or at the C-terminus of the  $\alpha$ -domain.

5

4. A peptide compound according to Claim 1, wherein said peptide compound is covalently bonded to a compound capable of providing a detectable signal.

10

5. A method of modulating cytolytic activity of a CTL, said method comprising:

15

combining a CTL with a peptide compound of at least 8 amino acids and not more than about 30 amino acids having a sequence differing by not more than two mutations from the polymorphic region of the C-terminal half of the  $a_1$  domain and the N-terminal half of the  $a_2$  domain of the host of said MHC restricted cells,

wherein said CTL cells are modulated by said peptide.

20

6. A method for inhibiting cytolytic activity of a CTL, said method comprising

25

combining CTL with a peptide compound of at least 8 amino acids and having a sequence coming within the extended sequence:

G S H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M aa<sup>99</sup> G C D  
 aa<sup>103</sup> G aa<sup>105</sup> D aa<sup>107</sup> R aa<sup>109</sup> L R G  
 aa<sup>113</sup> aa<sup>114</sup> Q aa<sup>116</sup> A Y D G;

30

wherein

aa<sup>94</sup> is T or I;

aa<sup>95</sup> is a non-polar aliphatic amino acid of  
 from 5 to 6 carbon atoms;  
 aa<sup>97</sup> is an aliphatic amino acid or W;  
 5 aa<sup>99</sup> is an aromatic amino acid;  
 aa<sup>103</sup> is a non-polar aliphatic amino acid of  
 from 5 to 6 carbon atoms;  
 aa<sup>105</sup> is P or S;  
 aa<sup>107</sup> is G or W;  
 10 aa<sup>109</sup> is L or F;  
 aa<sup>113</sup> is Y or H;  
 aa<sup>114</sup> is H, Q, D, N or R;  
 aa<sup>116</sup> is Y, D, S, F or H;

15 or

aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup>  
 aa<sup>66</sup> aa<sup>67</sup> K aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup>  
 aa<sup>77</sup> L aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A

20 wherein

aa<sup>55</sup> is E or K, particularly E;  
 aa<sup>62</sup> is G, Q, E or R, particularly R or G;  
 25 aa<sup>63</sup> is an acidic amino acid or amide thereof;  
 including E and N, particularly E;  
 aa<sup>65</sup> is Q, R or G, particularly Q or R;  
 aa<sup>66</sup> is I, N or K, particularly N or K;  
 aa<sup>67</sup> is an aliphatic neutral amino acid  
 30 including, V, M, S, C and Y, particularly V,  
 aa<sup>69</sup> is an aliphatic neutral amino acid  
 including, A, T and P, particularly A;  
 aa<sup>70</sup> is Q, H, S, N or K, particularly Q or H:



aa<sup>71</sup> is an aliphatic neutral amino acid  
 including S, A and T, particularly S;  
 aa<sup>74</sup> is D, Y or H, particularly D or H;  
 5 aa<sup>76</sup> is E or V;  
 aa<sup>77</sup> is D, S or N, particularly D;  
 aa<sup>79</sup> is R or G, particularly G;  
 aa<sup>80</sup> is T, I or N, particularly T or I;  
 aa<sup>81</sup> is an aliphatic non-polar amino acid  
 10 including L or A, particularly L;  
 aa<sup>82</sup> is R or L, particularly R;  
 aa<sup>83</sup> is G or R, particularly G, wherein said  
 CTL cells are inhibited by said peptide.

15 7. A method of sensitizing MHC restricted  
 cells to CTL, said method comprising:  
 combining MHC restricted cells with a peptide  
 compound of at least 8 amino acids and having a sequence  
 coming within the extended sequence

20 aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup>  
 aa<sup>66</sup> aa<sup>67</sup> K aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup>  
 aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A

25 wherein

aa<sup>55</sup> is E or K, particularly E;  
 aa<sup>62</sup> is G, Q, E or R, particularly R or G;  
 aa<sup>63</sup> is an acidic amino acid or amide thereof;  
 30 including E and N, particularly E;  
 aa<sup>65</sup> is Q, R or G, particularly Q or R;  
 aa<sup>66</sup> is I, N or K, particularly N or K;  
 aa<sup>67</sup> is an aliphatic neutral amino acid  
 including, V, M, S, C and Y, particularly V,

aa<sup>69</sup> is an aliphatic natural amino acid  
 including, A, T and P, particularly A;  
 aa<sup>70</sup> is Q, H, S, N or K, particularly Q or H;  
 5 aa<sup>71</sup> is an aliphatic neutral amino acid  
 including S, A and T, particularly S;  
 aa<sup>74</sup> is D, Y or H, particularly D or H;  
 aa<sup>76</sup> is E or V;  
 aa<sup>77</sup> is D, S or N, particularly D;  
 10 aa<sup>79</sup> is R or G, particularly G;  
 aa<sup>80</sup> is T, I or N, particularly T or I;  
 aa<sup>81</sup> is an aliphatic non-polar amino acid  
 including L or A, particularly L;  
 aa<sup>82</sup> is R or L, particularly R;  
 15 aa<sup>83</sup> is G or R, particularly G,  
 and wherein the MHC restricted cells become sensitized  
 to the CTL via the binding of said peptide.

8. A method for inhibiting cytolytic activity  
 20 of a CTL by irreversibly inhibiting the CTL, said method  
 comprising:

combining a CTL with a peptide compound of at  
 least 8 amino acids and having a sequence coming within  
 the extended sequence:

25 aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup>  
 aa<sup>66</sup> aa<sup>67</sup> K aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup>  
 R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A G S  
 H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M  
 30 aa<sup>99</sup> G C D aa<sup>103</sup> G aa<sup>105</sup> D aa<sup>107</sup> R aa<sup>109</sup> L R G  
 aa<sup>113</sup> aa<sup>114</sup> Q aa<sup>116</sup> A Y D G

wherein:

aa<sup>55</sup> is E or K;

- 5           aa<sup>62</sup> is G, Q, E or R;  
          aa<sup>63</sup> is an acidic amino acid or amide thereof;  
          aa<sup>65</sup> is Q, R or G;  
          aa<sup>66</sup> is I, N or K;  
          aa<sup>67</sup> is an aliphatic neutral amino acid;  
          aa<sup>69</sup> is an aliphatic neutral amino acid;  
          aa<sup>70</sup> is Q, H, S, N or K;  
          aa<sup>71</sup> is an aliphatic neutral amino acid;  
10          aa<sup>74</sup> is D, Y or H;  
          aa<sup>76</sup> is E or V;  
          aa<sup>77</sup> is D, S or N;  
          aa<sup>79</sup> is R or G;  
          aa<sup>80</sup> is T, I or N;  
15          aa<sup>81</sup> is an aliphatic no-polar amino acid;  
          aa<sup>82</sup> is R or L;  
          aa<sup>83</sup> is G or R;  
          aa<sup>94</sup> is T or I;  
          aa<sup>95</sup> is a non-polar aliphatic amino acid of  
20   from 5 to 6 carbon atoms;  
          aa<sup>97</sup> is an aliphatic amino acid or W;  
          aa<sup>99</sup> is an aromatic amino acid;  
          aa<sup>103</sup> is a non-polar aliphatic amino acid of  
          from 5 to 6 carbon atoms;  
25          aa<sup>105</sup> is P or S;  
          aa<sup>107</sup> is G or W;  
          aa<sup>109</sup> is L or F;  
          aa<sup>113</sup> is Y or H;  
          aa<sup>114</sup> is H, Q, D, N, or R;  
30          aa<sup>116</sup> is Y, D, S, F, or H

and wherein said peptide is linked with an agent which causes cytotoxicity; and wherein said peptide binds to said CTL cell.

9. A method for determining the presence of MHC restricted CTL's, said method comprising

5

contacting cells with a peptide compound of at least 8 amino acids and having a sequence coming within the extended sequence

10 aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> K  
 aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup>  
 aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A G S H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M aa<sup>99</sup> G  
 C D aa<sup>103</sup> G aa<sup>105</sup> D aa<sup>107</sup> R aa<sup>109</sup> LRG aa<sup>113</sup> aa<sup>114</sup> Q  
 aa<sup>116</sup> A Y D G

15

wherein:

aa<sup>55</sup> is E or K;  
 aa<sup>62</sup> is G, Q, E or R;  
 20 aa<sup>63</sup> is an acidic amino acid or amide thereof;  
 aa<sup>65</sup> is Q, R or G;  
 aa<sup>66</sup> is I, N or K;  
 aa<sup>67</sup> is an aliphatic neutral amino acid;  
 aa<sup>69</sup> is an aliphatic neutral amino acid;  
 25 aa<sup>70</sup> is Q H, S, N or K;  
 aa<sup>71</sup> is an aliphatic neutral amino acid;  
 aa<sup>74</sup> is D, Y or H;  
 aa<sup>76</sup> is E or V;  
 aa<sup>77</sup> is D, S or N;  
 30 aa<sup>79</sup> is R or G;  
 aa<sup>80</sup> is T, I or N, particularly T or I;  
 aa<sup>81</sup> is an aliphatic no-polar amino acid;  
 aa<sup>82</sup> is R or L, particularly R;  
 aa<sup>83</sup> is G or R;

aa<sup>94</sup> is T or I;  
 aa<sup>95</sup> is a non-polar aliphatic amino acid of  
 from 5 to 6 carbon atoms;  
 5 aa<sup>97</sup> is an aliphatic amino acid or W;  
 aa<sup>99</sup> is an aromatic amino acid;  
 aa<sup>103</sup> is a non-polar aliphatic amino acid of  
 from 5 to 6 carbon atoms;  
 aa<sup>105</sup> is P or S;  
 10 aa<sup>107</sup> is G or W;  
 aa<sup>109</sup> is L or F;  
 aa<sup>113</sup> is Y or H;  
 aa<sup>114</sup> is H, Q, D, N, or R;  
 aa<sup>116</sup> is Y, D, S, F, or H.

15 covalently joined to a compound capable of providing a  
 detectable signal; and  
 determining the presence of cells to which said  
 detectable signal compound is specifically bound.

20 10. A method according to Claim 9 wherein  
 said extended sequence is of the sequence:

25 G S H T (Y or L) I Q R M Y G C D V G S D W G F  
 L L R G Y H Q Y A Y D G

or

30 Q E G P E Y W D (G or R) (E or N) T (R or Q)  
 (K or N) V K A (H or Q) S Q T (H or D) R (V or E) (D, S  
 or N) L (G or R) (T or I) (L or A) (R or L) (G or R) Y Y  
 N Q S E A,

wherein the amino acids in the parentheses indicate that any of the amino acids in the parentheses may be present.

5

11. A substantially pure preparation of peptide compound selected from the sequences:

10

T L Q R M Y G C D V G S D W R F L R G,

M Y G C D V G S D W R F L R G Y,

M Y G C D V G S D G R F L R G Y,

G P E Y W D G E T R K V K A, and

15

G P E Y W D R N T R N V K A.

20

12. A pharmaceutical composition comprised of a peptide compound of at least 8 amino acids and having a sequence coming within the extended sequence

25

aa<sup>55</sup> G P E Y W D aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> K  
 aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q T aa<sup>74</sup> R aa<sup>76</sup> aa<sup>77</sup> L aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup>  
 aa<sup>82</sup> aa<sup>83</sup> Y Y N Q S E A G S H aa<sup>94</sup> aa<sup>95</sup> Q aa<sup>97</sup> M aa<sup>99</sup> G  
 C D aa<sup>103</sup> G aa<sup>105</sup> D aa<sup>107</sup> R aa<sup>109</sup> L R G aa<sup>113</sup> aa<sup>114</sup> Q  
 aa<sup>116</sup> A Y D G

wherein:

30

aa<sup>55</sup> is E or K;

aa<sup>62</sup> is G, Q, E or R;

aa<sup>63</sup> is an acidic amino acid or amide thereof;

aa<sup>65</sup> is Q, R or G;

aa<sup>66</sup> is I, N or K;

aa<sup>67</sup> is an aliphatic neutral amino acid;

aa<sup>69</sup> is an aliphatic neutral amino acid;

- 5 aa<sup>70</sup> is Q, H, S, N or K;  
aa<sup>71</sup> is an aliphatic neutral amino acid;  
aa<sup>74</sup> is D, Y or H;  
aa<sup>76</sup> is E or V;  
aa<sup>77</sup> is D, S or N;  
aa<sup>79</sup> is R or G;  
aa<sup>80</sup> is T, I or N;  
aa<sup>81</sup> is an aliphatic non-polar amino acid;  
10 aa<sup>82</sup> is R or L;  
aa<sup>83</sup> is G or R;  
aa<sup>94</sup> is T or I;  
aa<sup>95</sup> is a non-polar aliphatic amino acid of  
from 5 to 6 carbon atoms;  
15 aa<sup>97</sup> is an aliphatic amino acid or W;  
aa<sup>99</sup> is an aromatic amino acid;  
aa<sup>103</sup> is a non-polar aliphatic amino acid of  
from 5 to 6 carbon atoms;  
aa<sup>105</sup> is P or S;  
20 aa<sup>107</sup> is G or W;  
aa<sup>109</sup> is L or F;  
aa<sup>113</sup> is Y or H;  
aa<sup>114</sup> is H, Q, D, N or R;  
aa<sup>116</sup> is Y, D, S, F or H.

25

wherein said peptide compound is present in a  
pharmacologically effective dose in a pharmaceutically  
acceptable medium.

30

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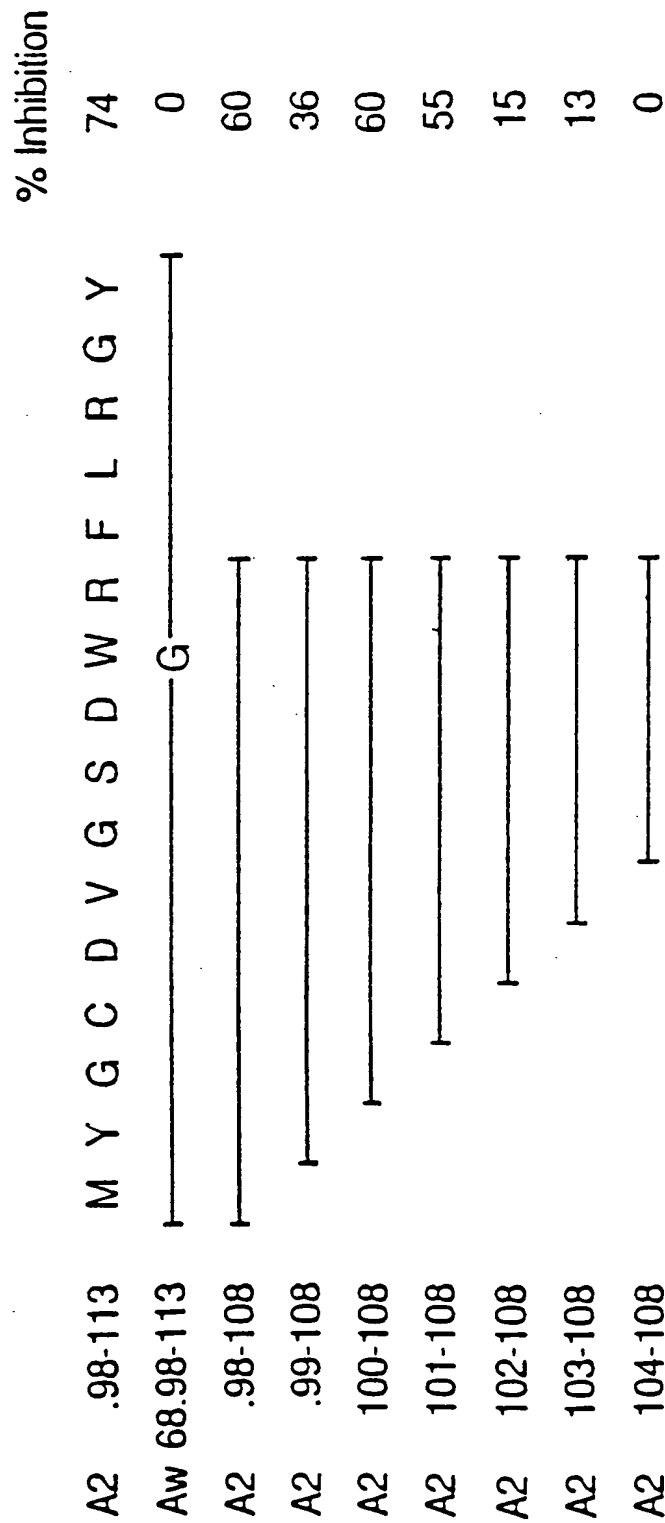


FIG. 1



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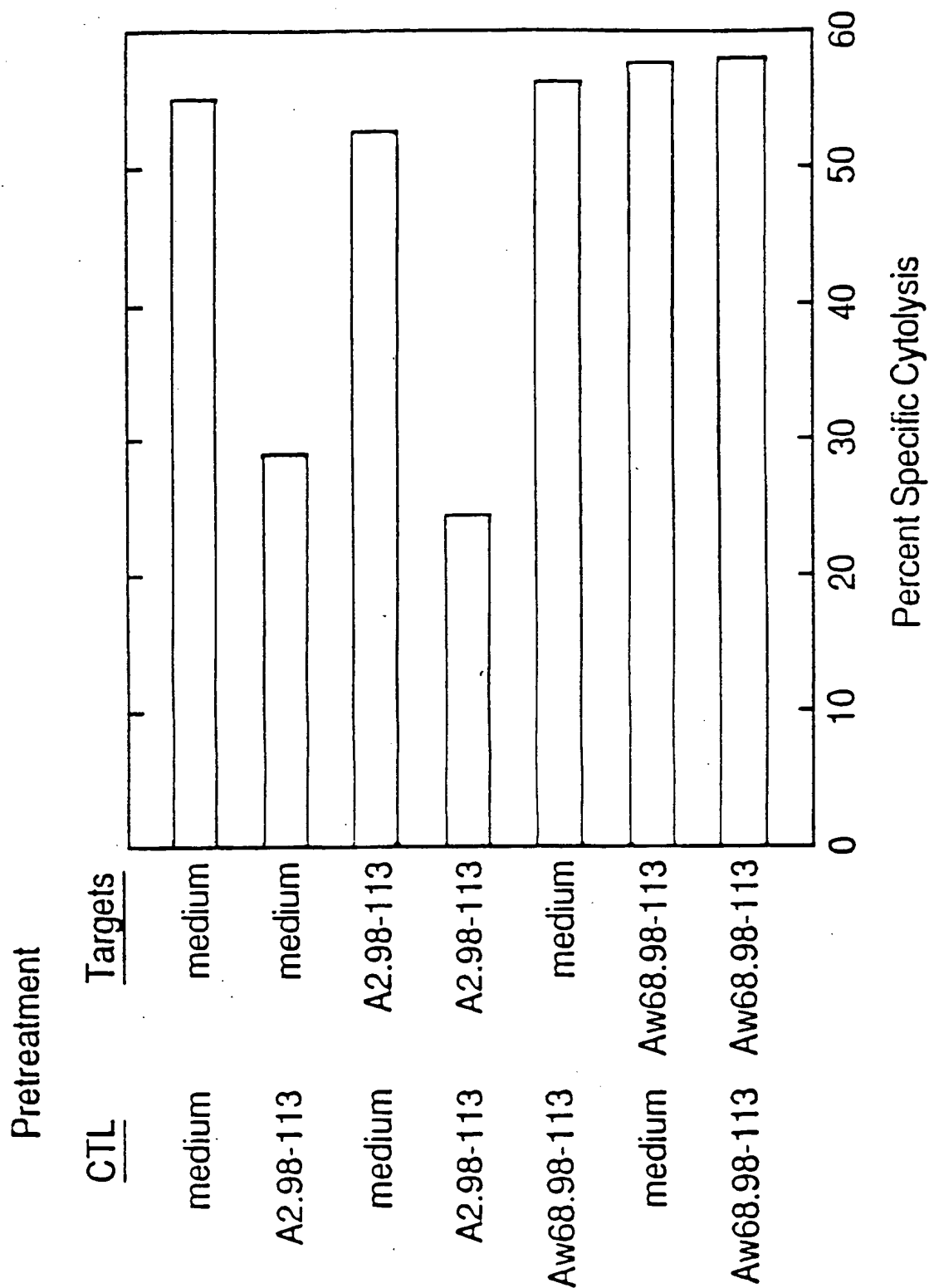


FIG. 2

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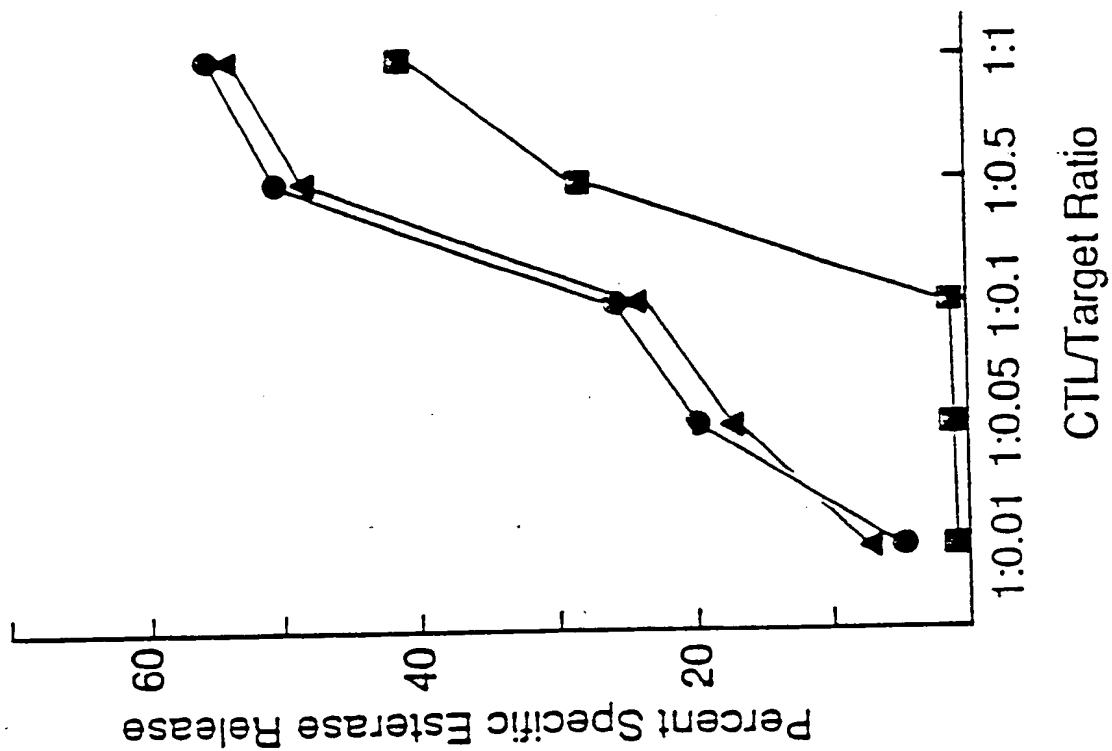


FIG. 3A

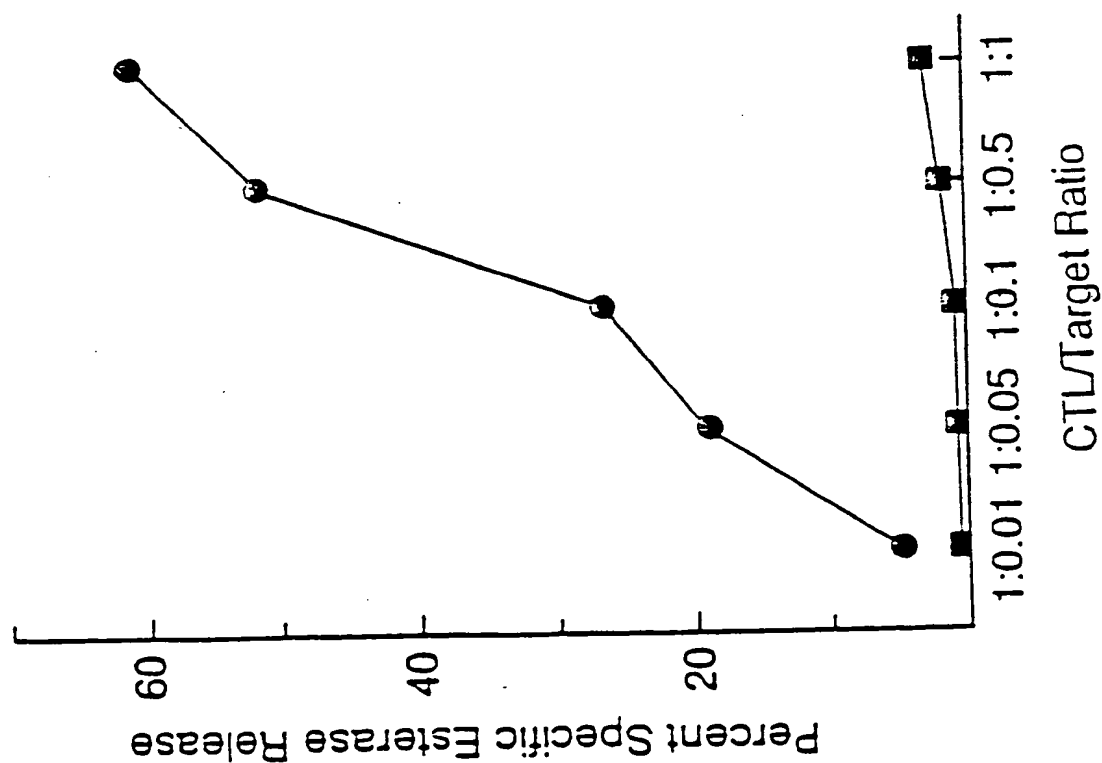


FIG. 3B



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FIG. 5A

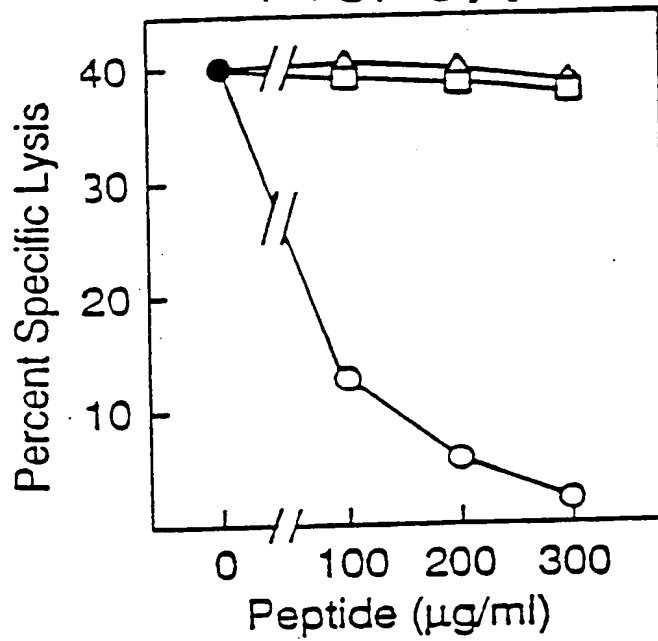


FIG. 5B

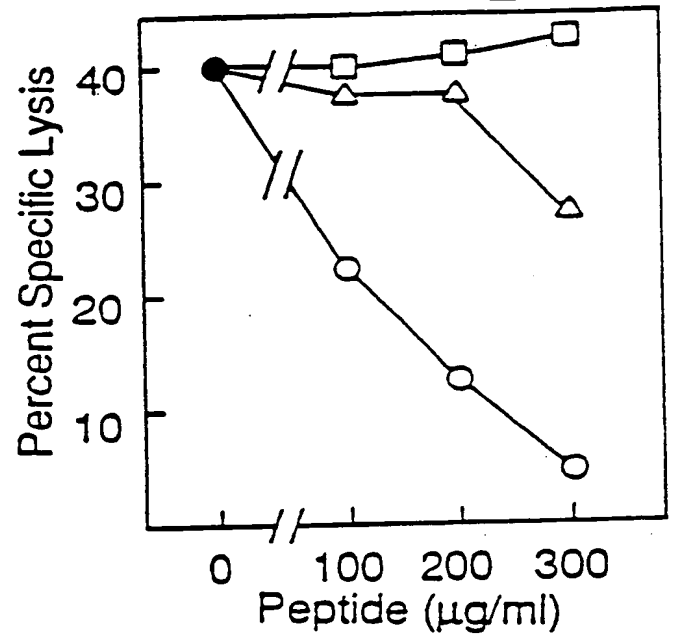


FIG. 5C

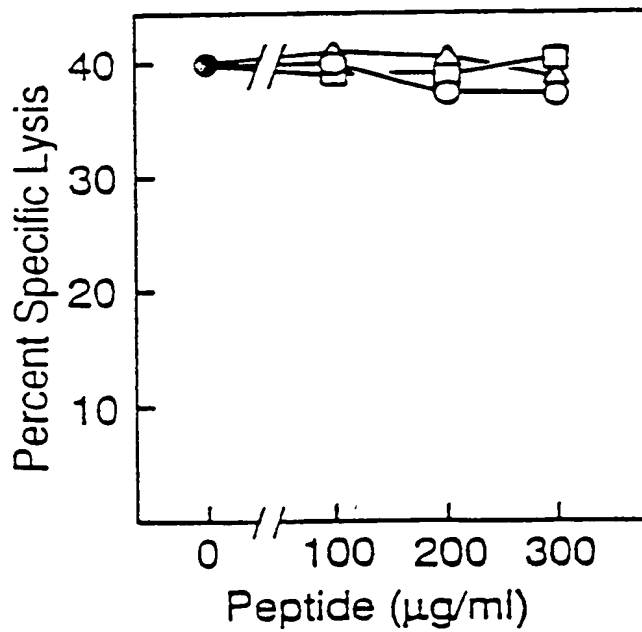
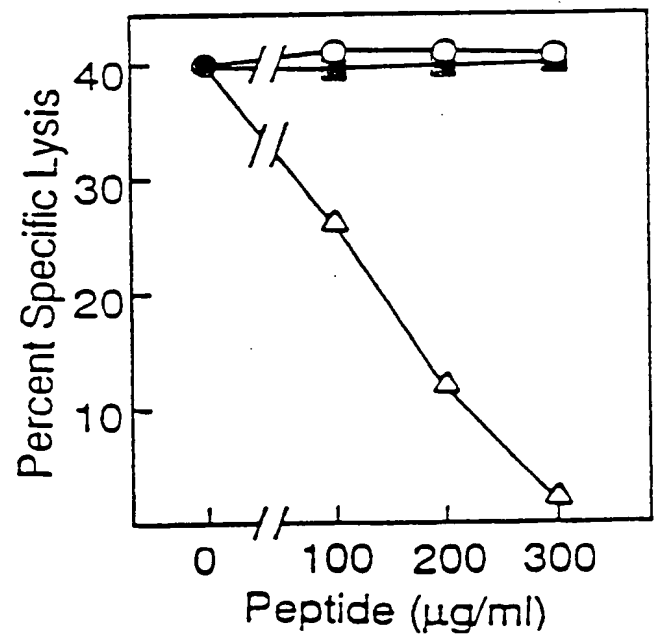


FIG. 5D



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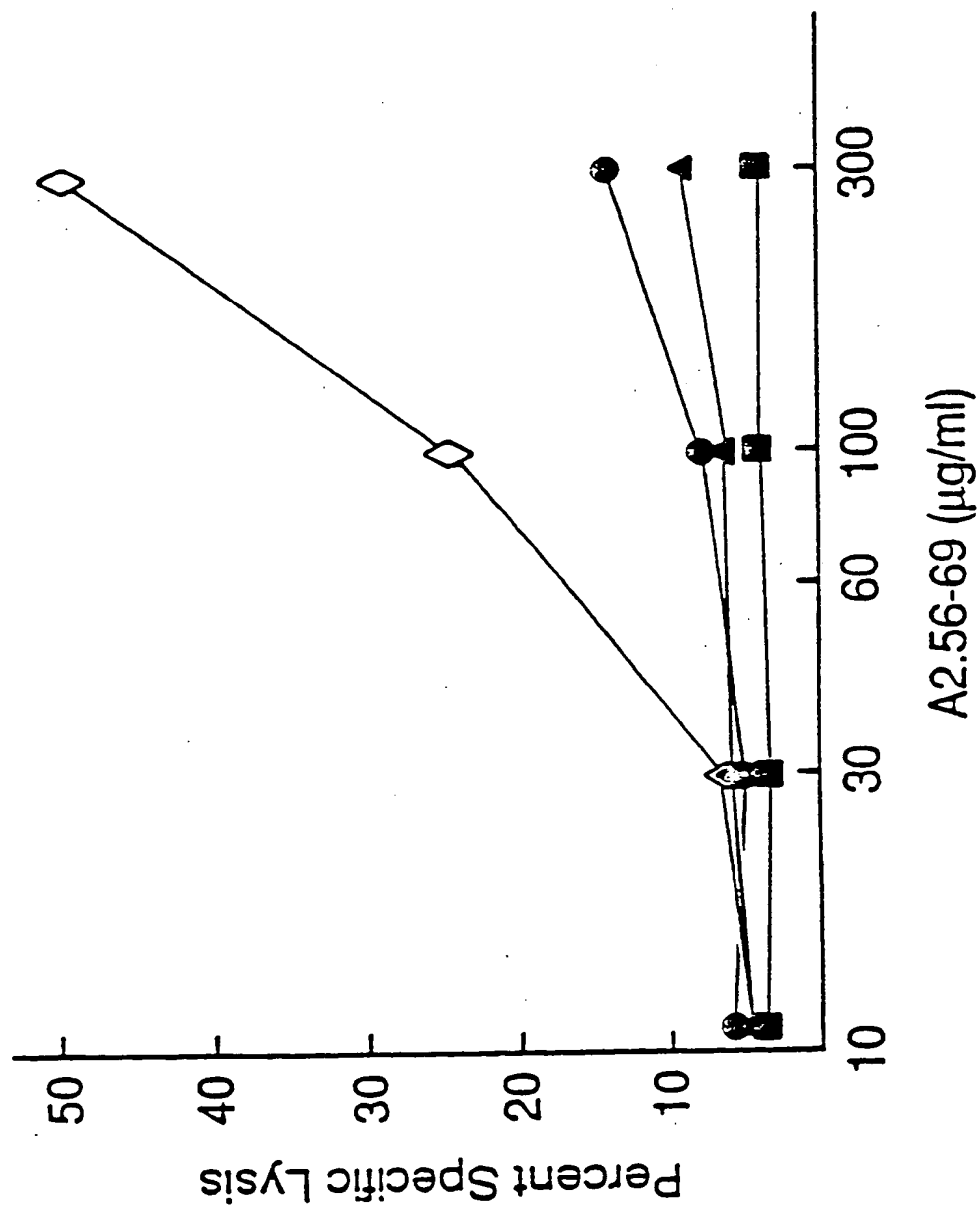
A2.56-69 ( $\mu\text{g/ml}$ )

FIG. 6

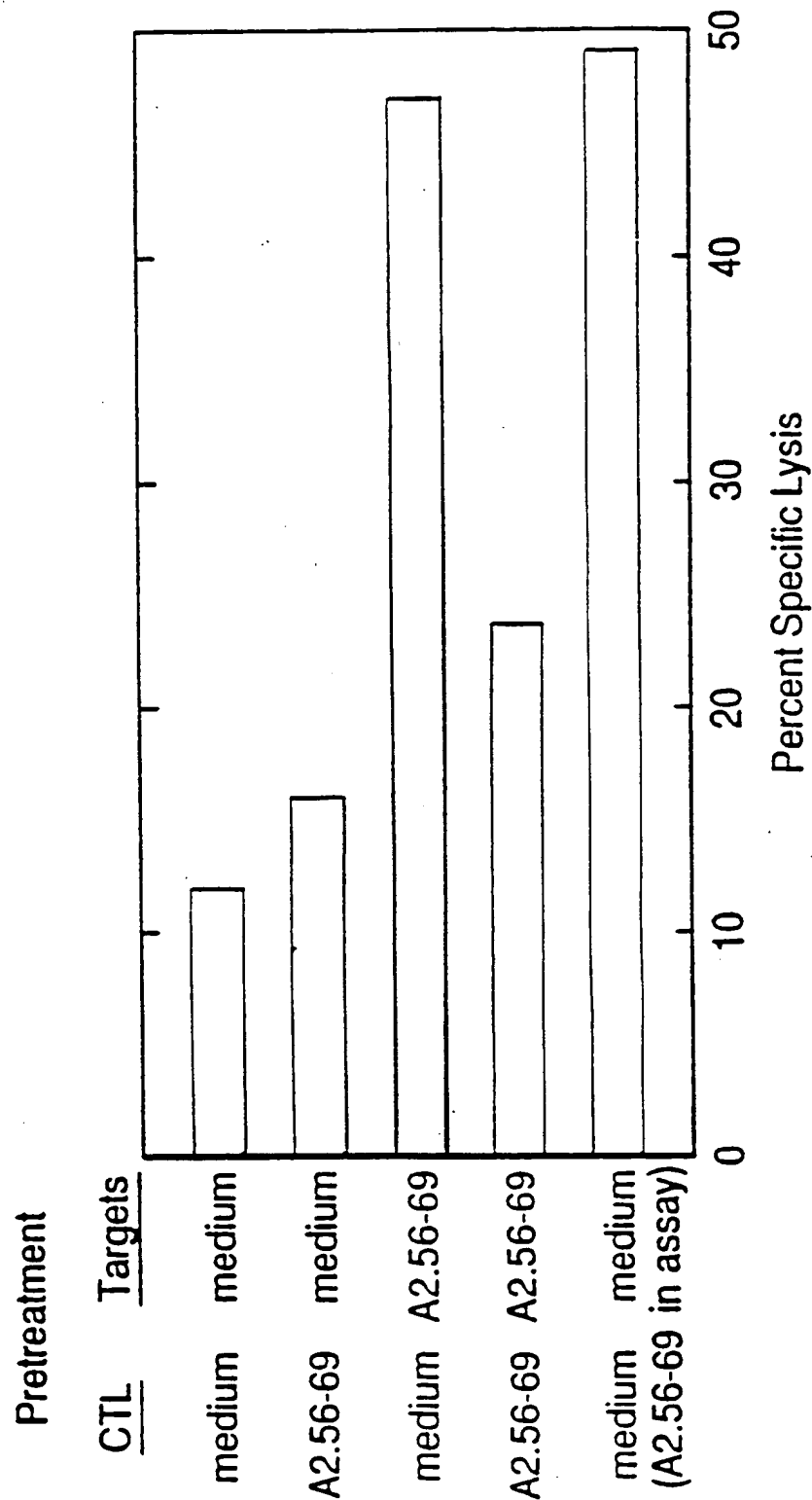


FIG. 7

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/00245

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>1</sup> According to International Patent Classification (IPC) or to both National Classification and IPC: IPC(4) C07K 7/06 7/08, 7/10; A61K 37/02; C12Q 1/04; US. CL. 530/324, 325, 326, 327, 328, 345, 802; 514/12, 13, 14, 15, 16; 435/29		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>4</sup>		
Classification System	Classification Symbols	
US.	530/324, 325, 326, 327, 328, 345, 802; 514/12, 13, 14, 15, 16; 435/29	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>4</sup>		
<b>CHEMICAL ABSTRACT AND BIOLOGICAL ABSTRACT ON LINE COMPUTER SEARCH</b>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>14</sup>		
Category <sup>9</sup>	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>
X	THE JOURNAL OF IMMUNOLOGY, VOLUME 134, ISSUED APRIL, 1985 (KOLLER ET AL), "CLONING AND COMPLETE SEQUENCE OF AN HLA-A <sub>2</sub> GENE: ANALYSIS OF TWO HLA-A ALLELES AT THE NUCLEOTIDE LEVEL" PAGES 2727-2733. SEE PAGE 2731 IN PARTICULAR.	1-2, 12
X	PROC. NATL. ACAD. SCI. USA, VOLUME 82, ISSUED NOVEMBER, 1985 (VEGA ET AL), "STRUCTURAL ANALYSIS OF AN HLA-B27 FUNCTIONAL VARIANT: IDENTIFICATION OF RESIDUES THAT CONTRIBUTE TO THE SPECIFICITY OF RECOGNITION BY CYTOLYTIC T LYMPHOCYTES", PAGES 7394-7398. SEE P. 7396, 7397, 7398 IN PARTICULAR.	1-2, 5-7, 12
P, X	CHEMICAL ABSTRACTS, (COLUMBUS, OHIO, USA), VOLUME 106, ISSUED 1987, (PARHAM ET AL), "INHIBITION OF ALLOREACTIVE CYTOTOXIC T LYMPHOCYTES BY PEPTIDES FROM THE $\alpha_2$ DOMAIN OF HLA-A <sub>2</sub> , SEE PAGE 516, COLUMN 1, THE ABSTRACT NO. 154384x, NATURE, 1987 325 (6105) 625-8, (ENG.).	1-2, 5-6 11-12
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>12</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATE</b>		
Date of the Actual Completion of the International Search <sup>1</sup>		Date of Mailing of this International Search Report <sup>1</sup>
07 MARCH 1988		04 APR 1988
International Searching Authority <sup>1</sup>		Signature of Authorized Officer <sup>10</sup>
ISA/US		CHRISTINA CHAN <i>Christina Chan</i>

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